TEST SECRETION IN TWO SPECIES OF FOLLICULINA

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Introduction

The fact that *Folliculina* passes through a free-swimming stage at some point in its life cycle was recognized by Wright (1859) and by Claparède and Lachmann (1858–61). The former author observed the transformation of the free-swimmer of *F. producta* into the adult form, but did not discover the mode of origin of the "larva" nor any details of the metamorphosis. For this reason these observations were later questioned by Stein (1867), but they are completely confirmed by more recent work. That the larvae are formed as the result of cell division was pointed out by Möbius (1887), but the presence of a larva and an adult lying side by side in a single test he interpreted as a case of longitudinal division. In his figures he even shows an "umbilical cord" projecting laterally from the larva. *Folliculinas* do not, however, depart from the general rule of transverse division in ciliates as was pointed out by Sahrhage (1917). Andrews (1920) has shown that free-swimming larvae may also result from the dedifferentiation of an adult form.

Test secretion and metamorphosis are described by Penard (1919) for *F. boltoni,* a freshwater species. Too few details are given to decide whether or not this species passes through the same stages as are described for *F. producta* by Andrews (1923) or for *F. simplex* (previously called *F. ampulla*) by Fauré-Fremiet (1932). Observations on *F. aculeata* and *F. elegans* indicate that the process of test formation is somewhat different in these species. During the summer of 1936 at Woods Hole, while examining fresh preparations for specimens of *Folliculina,* a form was discovered of which no description could be found. The posterior part of the body resembled that of a *Folliculina* seen on the slide, but the anterior portion was drawn out into one long, slender, flexible process bearing membranelles only at the end. Circumstantial evidence pointed to the conclusion that these forms represented a stage in the life history of *Folliculina.* Definite proof of this theory was lacking, since the larvae did not remain in good condition long enough for complete transformation to take place. This stage has evi-

dently been noted by Fauré-Fremiet (1936), but his descriptions are not detailed. In order to obtain more complete evidence concerning the significance of this stage in the life cycle, observations were resumed in 1939. A complete life history can now be given placing this stage in its proper sequence.

The classification of the members of the family Folliculinidae is still in a somewhat unsatisfactory state in spite of the fact that a number of investigators have given the matter a great deal of attention. The separation of the genus *Folliculina* into a large number of species on the basis of characteristics which are subject to considerable variation seems to be the rule. For this reason the classification given by Fauré-Fremiet (1936), in which several species which have a number of common characteristics are combined into a single species, is to be preferred. Two species, as described by this author, *F. aculeata* and *F. elegans,* seem to prevail at Woods Hole. The two are alike in many ways; the chief differences are in size, the presence or absence of pointed tips on the peristomeal lobes and in the pigmentation of the animal and of the test. Since all of these traits are subject to variation, so that one species may resemble the other very closely, it was difficult to determine which species was under observation. Certain organisms, however, presented all the criteria of one species or the other. Since the greater number of individuals observed were of a paler color and possessed the pointed peristomeal lobes characteristic of *F. aculeata,* this species is figured. The only point in which they differed from the descriptions of Fauré-Fremiet was in the color of the test which was often colorless or faintly blue. Both species have an ovoid nucleus, but, as in *F. boltoni,* it is often notched or bi-lobed.

Methods

The organisms were collected by placing glass slides in crystallizing dishes containing quantities of the hydroid *Tubularia* and leaving them for several days in running sea water. The slides were then removed and placed in Petri dishes containing sea water. Observations were made with a binocular dissecting microscope, with the 16 mm. objective of a compound microscope or with a water immersion lens (40 ×). In order to maintain the organisms in good condition over periods of several hours, a stream of fresh sea water was run into the dish while on the stage of the microscope. The overflow was carried off by means of an inverted siphon. Temperature readings were regularly recorded and during the time that the observations were being made the temperature of the sea water varied from 21° C. to 24° C.

Observations

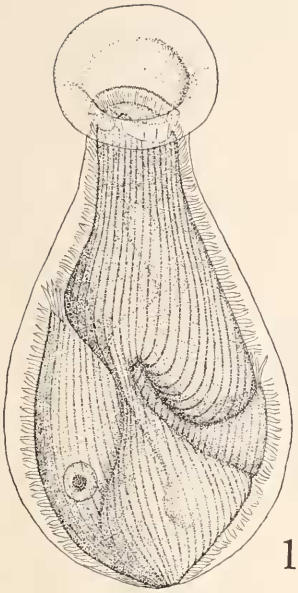
The complete life history of a larva resulting from cell division, from the time of departure from the test containing the sister cell until the adult form was attained, was followed for a number of individuals. In some cases free-swimming larvae were found and their subsequent history observed. In a few cases it was found that adult forms dedifferentiated into the larval form, left the test and settled down to secrete a new test.

After cell division (Fig. 1) the anterior individual, which will eventually leave the test as a free-swimming larva, remains for from 30 to 60 minutes in the test contracting and extending beside its sister cell, which remains attached and which has already begun the metamorphosis into the adult form. At each extension of the larva the anterior end is projected farther and farther out of the neck of the test until finally the whole organism is free. The swimming stage (Fig. 2) may last for from 15 to 90 minutes, during which time the larva swims slowly along the substratum or more rapidly near the surface of the water in the dish. At intervals it pauses to contract and extend itself at one spot and then swims on. Just before the larva settles down to secrete its test it may be seen to repeat this process of contraction and elongation a number of times in a single spot, changing direction each time it contracts until it has described a complete circle at least once and sometimes several times. It then flattens itself out on the substratum and its outlines become very irregular (Fig. 3). Occasionally larvae were seen secreting tests attached to the surface film of the water as described by Wright in 1859. This flattened stage lasts for several minutes, after which time it is difficult to dislodge the organism from the slide even with a fairly strong stream of water. It is at this time that a broad layer of cement substance is being secreted which will serve to attach the test to the substratum. During this stage and for the entire time that the test is being secreted the anterior end of the animal is raised above the rest of the body.

Having attached itself firmly, the animal now assumes a more regular ovoid form, becoming thicker and rounder (Fig. 4). Soon granules may be seen around the body among the cilia. These collect all around the periphery and harden to form the bottle-shaped part of the test.

Figs. 1–4. *Folliculina aculeata.* × 300.

Fig. 1. Late division stage. Macronucleus not yet completely divided.
Fig. 2. Free-swimming larva.
Fig. 3. Larva flattened out on the substratum during the process of cement secretion.
Fig. 4. Beginning of the secretion of the test.



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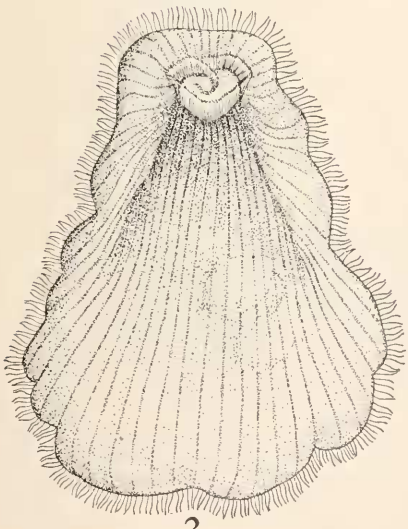

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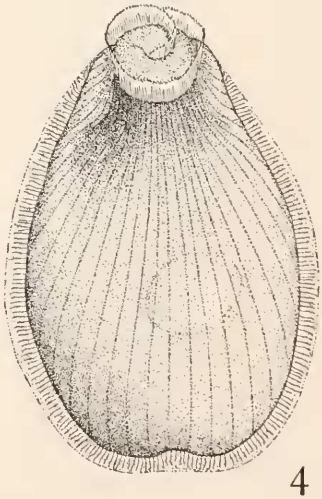

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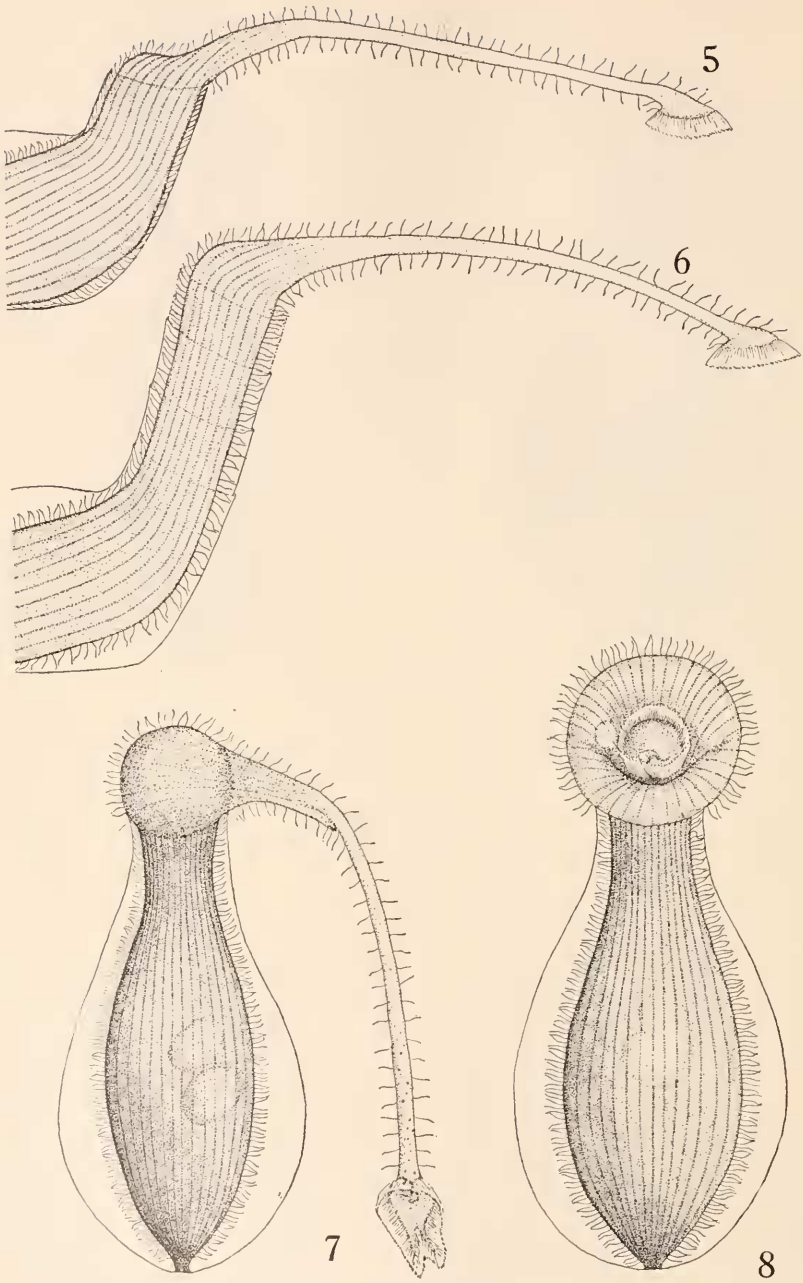

FIGS. 5–8. *Folliculina aculeata.* × 300.

FIG. 5. Side view of larva with long proboscis at the beginning of secretion of the neck of the test.

FIG. 6. Same as Fig. 5. Neck secretion nearly completed.

FIG. 7. Same as Fig. 5. Top view.

FIG. 8. Beginning of secretion of the collar of the test.

The upraised anterior portion of the body secretes the base of the neck of the test. As the formation of the body of the test is completed, the anterior end of the animal elongates to form a proboscis-like projection

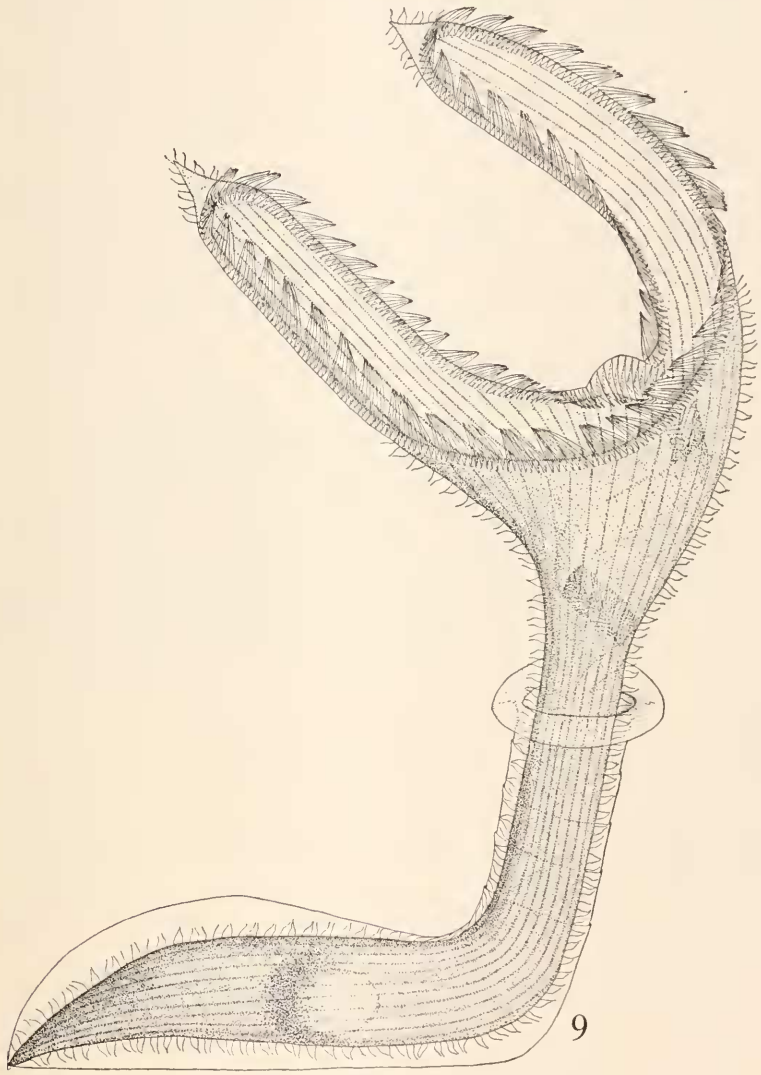

FIG. 9. Adult *Folliculina aculeata*. × 300.

of about the same length as the body. Upon the end of this proboscis the membranelles are borne (Figs. 5, 7). The proboscis is seen to sweep about in circles and while it is present the neck of the test becomes

longer and the spiral rings are laid down (Fig. 6). This stage is observed about 50 to 75 minutes after the larva has become attached and lasts about 70 to 100 minutes.

It is difficult to state whether or not this stage has previously been described. Penard (1919) states that Lachmann observed the fixation of a larva of *F. elegans* "after which . . . at the anterior end a membranous extension appeared, which I should be disposed to consider as moribund phenomena" (p. 312). Upon referring to the original paper this stage is described as presenting an "épanouissement membraneux" (p. 219). This seems clearly to refer to the next stage to be described in this cycle. It is improbable that this is an abnormality in the development, since this stage was observed in every larva whose development was followed. In the metamorphosis of *F. producta* and *F. simplex* this stage is apparently lacking (Andrews, 1923; Fauré-Fremiet, 1932). The figures of Fauré-Fremiet (1936) cannot be definitely identified with this stage.

Upon completion of the tubular part of the neck of the test, the proboscis is retracted and a double fold of cytoplasm is extended around the opening to form a collar (Fig. 8). During the 55 to 115 minutes that this stage lasts the collar of the test is secreted. When this has been finished the cytoplasm frees itself from the rim of the collar and the ragged edges are withdrawn into the test. In this contracted state the animal remains for from 3 to 5 hours. The changes occurring during this time are described in detail by both Andrews and Fauré-Fremiet and seem to be essentially similar in all forms. At the end of this period the animal protrudes from the test the long peristomeal lobes characteristic of the adult form (Fig. 9) The entire process of test secretion and morphogenesis may take from 4½ to 8¾ hours.

A single small larva of a yellow color, probably *F. viridis,* was discovered on the slide in the early stages of test secretion and was followed through to the adult stage. The development followed that of *F. aculeata* and *F. elegans,* although the proboscis was relatively shorter than in these species and the peristomeal lobes of the adult were smaller and more rounded.

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Summary

1. Test secretion and metamorphosis in *Folliculina aculeata* and *Folliculina elegans* are described.

2. These processes may be divided into the following six stages:

a. The free-swimming stage.
b. The stage of cement secretion.
c. The secretion of the body of the test.
d. The secretion of the neck of the test, during which process the animal puts forth a long, proboscis-like projection. This stage is, as far as is known, peculiar to *F. aculeata, F. elegans* and *F. viridis* and has not been described before in detail.
e. The secretion of the collar of the test.
f. Formation of the peristomeal lobes characteristic of the adult.

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